STUDIES IN THE METABOLISM OF APPLES.

IV. FURTHER STUDIES IN THE RESPIRATORY METABOLISM OF GRANNY SMITH APPLES, WITH SPECIAL REFERENCE TO THE IMPORTANCE OF OXYGEN SUPPLY.

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(Ten Text-figures.)

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Introduction.

The second paper of this series (Hackney, 1943) was concerned with the relationships between changes in the composition of the internal atmosphere and changes in the respiratory activity of mature Granny Smith apples. It appeared from the data from apples which had been stored for less than three or four months that the rate of output of carbon dioxide was governed by the internal oxygen concentration, and that this in turn was limited by the resistance of the skin of the fruit to the diffusion of oxygen from the surrounding air. In fruits which had been stored for more than five months the rate of output of carbon dioxide was not generally governed by the internal oxygen concentration.

In calculating the resistance of the skin to the passage of oxygen, the present writer assumed that the volume of oxygen utilized in respiration was approximately equal to that of carbon dioxide given off. During 1943, a new technique was adopted by means of which the rate of oxygen uptake was determined as well as the rate of carbon dioxide output. Two long series of experiments were carried out: the first on immature fruits which had not been in cool store; the second on apples of commercial maturity after various periods of cool storage. The results are presented in this paper.

In addition to the experiments on the respiration of apples in air, several experiments were carried out on the respiration of fruits in an atmosphere of pure oxygen. The results of these investigations add to the evidence of the importance of oxygen supply in the metabolism of stored apples.

Materials and Methods.

All the apples were obtained from the same orchard at Orange, New South Wales. As the 1943 crop was very light the trees had not been thinned.

The immature apples used in the first series of experiments were taken from two selected trees: a sample of six fruits was taken from the trees each fortnight. On the day after picking, the fruits were put in a room maintained at 18·3°C., and the first observations were made twenty-four hours later. Subsequent observations were made every second day for two weeks. Great variability was observed in the respiratory behaviour of immature fruits. Several unsuccessful attempts were made to discover the cause of this variability. The first of these was an experiment in which four samples of six fruits were taken, two samples from each tree. One sample from each tree was composed of fruits which had developed in proximity to few leaves; the other sample was composed of fruits which had developed in proximity to many leaves. There was no difference in the variability in any of these samples. Within a sample there was no correlation between size of fruit and rate of respiration, whether expressed as milligrams per 10 Kilograms per hour or as milligrams per fruit per hour. A second experiment was designed to discover any differences (*a*) between fruits from the top

of the tree and those from the bottom of the same tree, and (*b*) between fruits from the part of the tree most exposed to sunlight and those from the most shaded part of the same tree. Again there was no difference in the mean level of respiration or variability in any of the four samples taken. It appears that the variability was not due to differences between the two trees nor to differences in position on the tree.

The apples used in the second series of experiments were of commercial maturity, picked on 30.iv.43. They were stored at 1°C. A sample of five fruits was withdrawn from store each fortnight and its subsequent respiratory behaviour was observed at 18·3°C. On most of the samples the observations were continued for at least four weeks, but in some of the later samples the fruits deteriorated within two weeks after removal from store, and observations were discontinued.

One experiment sought to discover whether there were differences in respiratory behaviour between mature apples from the two selected trees mentioned above (from which the immature fruits were taken) and apples which were taken from other trees in the same orchard to be used in the second series of experiments. No difference was detected. Variability between mature individuals was less marked than between immature individuals.

In the two long series of experiments the rates of oxygen uptake and carbon dioxide output were obtained with a respirometer of the type described by Sykes (1944). The first observations were begun about twenty-five hours after removal from store; assuming that the specific heat and thermal conductivity of apples approximate to those of water, calculations indicate that after this period the temperature at all points in the apple should be within 0·01°C. of the outside temperature, 18·3°C. Such slight differences in temperature would not have caused inaccuracy in the respirometer readings.

In the series of experiments with apples in pure oxygen this gas was passed through the apparatus from a cylinder. It was first blown through baryta water and shown to contain no carbon dioxide; it was then passed through the respiration vessels and the rate of carbon dioxide output was measured by the Pettenkofer method. Rates of oxygen uptake were not obtained.

The concentrations of oxygen and carbon dioxide in the internal atmosphere were measured by the method described in the first paper of this series. (Trout *et al.* 1942).

EXPERIMENTAL RESULTS. A.—IMMATURE FRUITS AT 18·3°C.

Table 1 shows date of picking, date of insertion of gas-sampling pipette,* date of first observation and date of final observation for each of the nine samples taken.

TABLE 1.
Immature Granny Smith Apples of the 1941–1942 Season.

Sample No.	Date of Picking.	Date of Setting Up.	Date of First Observation.	Date of Final Observation.
1a	7.xii.42	8.xii.42	9.xii.42	21.xii.42
2a	21.xii.42	22.xii.42	23.xii.42	4.i.43
3a	4.i.43	5.i.43	6.i.43	18.i.43
4a	18.i.43	19.i.43	20.i.43	26.i.43
5a	15.ii.43	16.ii.43	17.ii.43	1.iii.43
6a	1.iii.43	2.iii.43	3.iii.43	16.iii.43
7a	15.iii.43	16.iii.43	17.iii.43	30.iii.43
8a	29.iii.43	30.iii.43	31.iii.43	13.iv.43
9a	13.iv.43	14.iv.43	15.iv.43	29.iv.43

Respiration Rates.—When rates of oxygen uptake and carbon dioxide output were calculated in mg./10Kg./hr., those of the earlier samples were very much higher than the corresponding rates for mature fruits. The mean initial rates decreased as development proceeded; and in samples 7, 8 and 9, they were very much the same as those of the mature samples which followed (see Fig. 1). However, it has been suggested (Kidd, 1935; Clendenning, 1942) that, when comparisons are to be made between fruits at

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* To sample the internal atmosphere of the fruit, i.e., the atmosphere in the intercellular space system.

various stages of development, respiration rates should be calculated in units of gas taken up or given off per fruit, rather than per unit weight. Cell division ceases when the apple is about 3 cm. in diameter (Smith, 1940); further increase in size is due to cell enlargement, which is the result of enlargement of the vacuoles rather than of increase in the volume of the protoplasmic contents of the cell. Thus it is possible that for a single cell, the amount of actively respiring material is the same in an immature apple weighing 30 gm. as in a mature apple weighing 150 gm.
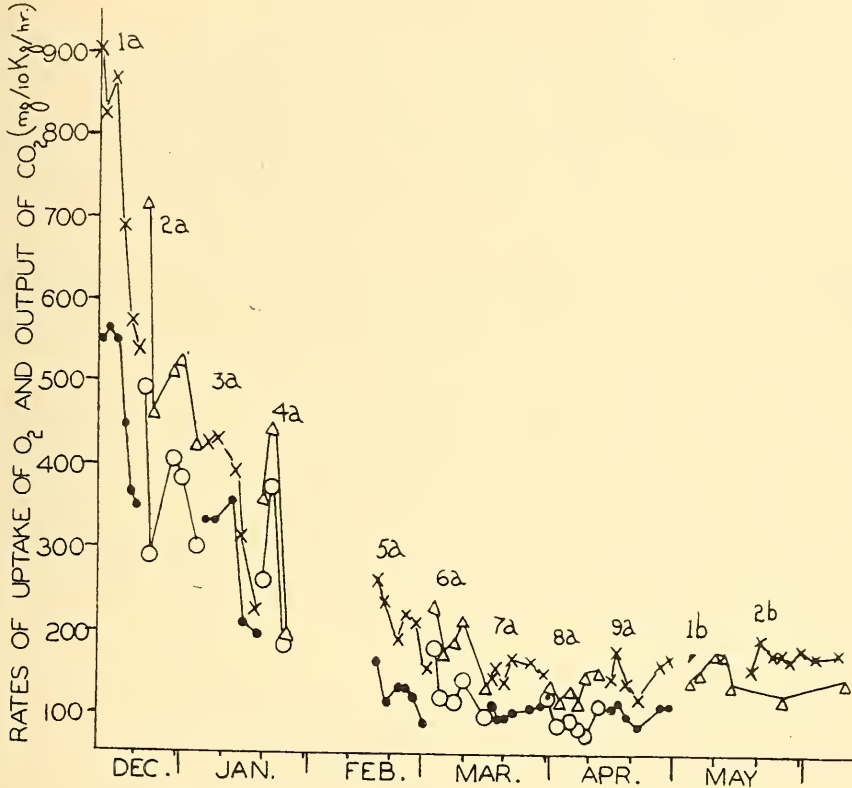

Fig. 1.—Graph showing the mean rates of oxygen uptake and carbon dioxide output per unit weight for each of the samples of immature Granny Smith apples (1a to 9a inclusive), and the mean rates of carbon dioxide output for the first two mature samples (1b and 2b). Values for rate of oxygen uptake are indicated by black spots and white circles for alternate samples; values for rate of carbon dioxide output are indicated by crosses and triangles for alternate samples.

Of the immature samples studied, number 1a (average diameter 3·2 cm.; average weight 17 gm.) was the only one in which cell division might not have ended. In sample 2a (average diameter 4·1 cm.; average weight 32 gm.) cell division can be presumed to have ended. Figure 2 shows mean initial rates of oxygen uptake and carbon dioxide output, calculated in units per fruit, for all the samples of mature and immature fruits. The pronounced difference in the behaviour of immature and mature fruits, which was apparent when respiration rates were calculated in units of gas taken up or given off per unit weight (Fig. 1), is not evident when they are calculated in units per fruit. The mean initial rates did not change significantly as development proceeded (Fig. 2). N. O'Grady, working with immature Granny Smith apples in 1941, found that the rate of carbon dioxide output decreased as development proceeded when calculated per unit weight, but appeared to increase when calculated per fruit.*

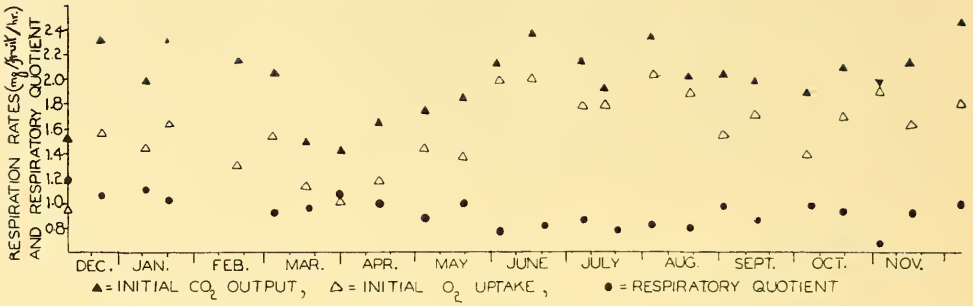* Unpublished data, Department of Botany, University of Sydney.

Fig. 2.—Graph showing the mean initial rates of oxygen uptake per fruit, carbon dioxide output per fruit, and mean initial values for respiratory quotient for all the samples of immature and mature Granny Smith apples. The first of the mature samples was taken in the first week of May, 1943.

The rates of uptake of oxygen and output of carbon dioxide for individual fruits generally fell, but fluctuations occurred. It is unlikely that the greatest of these fluctuations were analogous to the 'climacteric rise', since they were not accompanied by any signs of ripening. They were probably analogous to the 'pre-climacteric humps' described by Krotkov (1941).

The respiratory quotient was approximately 1 throughout the period of observation.

Composition of the Internal Atmosphere.—Figure 3 shows the mean concentrations of oxygen and carbon dioxide present in the internal atmospheres of the various samples. In the earliest samples the concentration of oxygen was very high (about 20%) and that of carbon dioxide was low (about 1%). In later samples the concentration of oxygen was about 2% lower, and that of carbon dioxide was about 1% higher, than in earlier samples. Compared with the values obtained later for mature fruits, the concentration of oxygen in immature fruits was always high and that of carbon dioxide low.
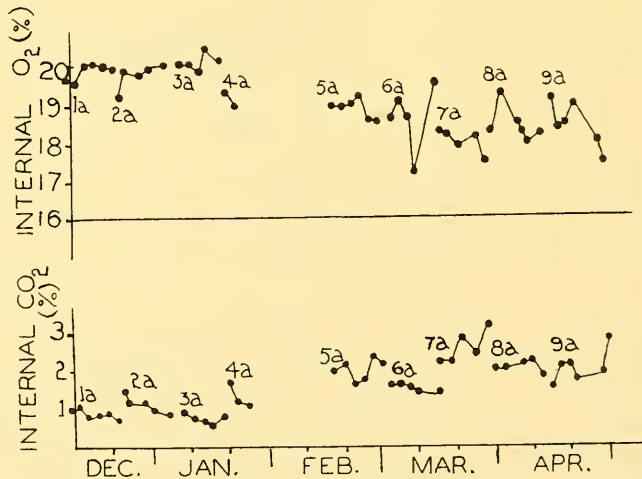

Fig. 3.—Graphs showing the mean internal concentrations of oxygen and carbon dioxide for each of the samples of immature Granny Smith apples taken in 1942-43.

Resistance of the Skin to Gaseous Diffusion.—It has been suggested (Wardlaw and Leonard, 1936) that the fall in respiration rate as development proceeds might be due in some measure to the reduction of the surface-volume ratio (s/v) which takes place as the fruit enlarges. The immature fruit is almost spherical and the reduction in s/v consequent upon each small increase in diameter is greater in the early samples; in the

later samples the reduction is smaller (Fig. 4). This means that as the apple enlarges it becomes more difficult for the oxygen required for respiration to enter the fruit and for the carbon dioxide produced to escape. This might be expected to result in a decreased concentration of oxygen and an increased concentration of carbon dioxide in the internal atmosphere, as development proceeds, and these have, in fact, been observed (Fig. 3). However, a study of the changes in the resistance of the skin to
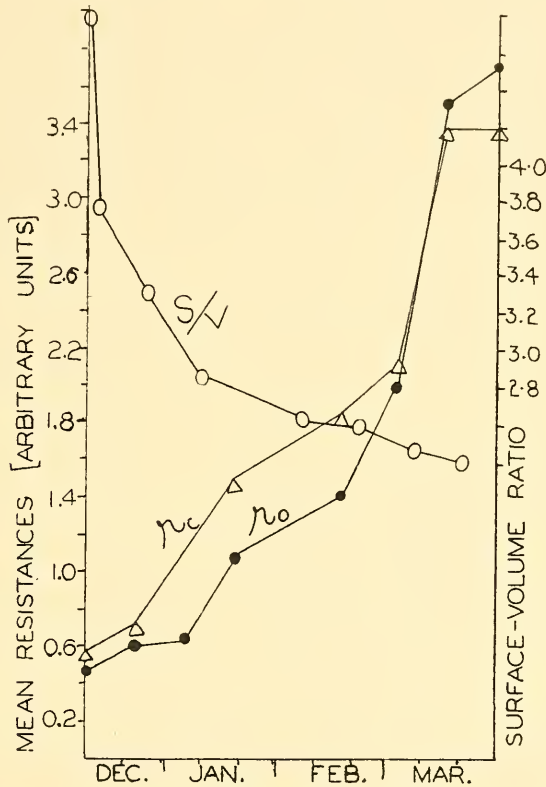

Fig. 4.—Graph showing the surface-volume ratio (s/v) and the mean initial values for resistances (per unit surface) to the diffusion of oxygen (r_o) and carbon dioxide (r_c) in each of the samples of immature apples taken in 1942-43.

gaseous diffusion shows that reduction in s/v from sample to sample is not the complete explanation of the changes which occur. Previous papers (Trout *et al.*, 1942; Hackney, 1943) have dealt exclusively with mature fruits which were of such a volume that small differences in size did not cause appreciable changes in the value of s/v. The formulae used for the calculation of the resistances of the skin per unit area to the passage of oxygen (r_o) and of carbon dioxide (r_c) are:

$$r_o = \frac{\delta O + 0 \cdot 2\, \delta N}{qR} \text{ and } r_c = \frac{\delta C}{qR},$$

where R is the rate of production of carbon dioxide in c.c. given off per sq. cm. per unit of time, qR is the corresponding amount of oxygen absorbed, and δO, δN and δC are the differences in concentrations of oxygen, nitrogen and carbon dioxide, respectively, between the internal atmosphere of the fruit and the external atmosphere. These formulae give r_o and r_c in arbitrary units. For comparisons between mature fruits, where s/v does not vary greatly with small variations in size, it is not necessary to calculate R in c.c. per unit surface; the rate of output of carbon dioxide per unit weight can be used instead. This has been done in previous papers and will be done again in this paper in the section on mature fruits. It is necessary to calculate rates of uptake

and output of gases in units per unit surface in order to compare skin resistances in immature fruits where s/v is changing rapidly. This method gives the resistance of the skin per unit surface and eliminates the surface-volume ratio. Fig. 4 shows that both r_o and r_c increased as development proceeded in the immature fruits of 1942–43. Thus there is another factor, apart from changes in surface-volume ratio, causing the resistance of the fruit to gaseous diffusion to increase as development proceeds.

It must be remembered that the figures for r_o and r_c in immature apples are in different units from those which will be presented later for mature apples; the two sets of figures may only be compared qualitatively although different values within each group, being in the same units, may be compared quantitatively.

EXPERIMENTAL RESULTS.   B.—MATURE FRUITS AT 18·3°C.

Table 2 shows date of removal from store and insertion of gas-sampling pipette, date of first observation, date of final observation, and number of weeks in cool store, for each of the fifteen samples taken during 1943.

TABLE 2.

Sample No.	Date of Removal from Store.	Date of First Observation.	Date of Final Observation.	Number of Weeks in Cool Store.
1b	3.v	4.v	5.vii	0
2b	18.v	19.v	20.vii	2
3b	1.vi	2.vi	22.vii	4
4b	15.vi	16.vi	22.vii	6
5b	6.vii	7.vii	17.viii	9
6b	20.vii	21.vii	26.viii	11
7b	2.viii	3.viii	9.ix	13
8b	18.viii	19.viii	28.x	15
9b	31.viii	1.ix	21.x	17
10b	14.ix	15.ix	28.x	19
11b	5.x	6.x	18.xi	22
12b	19.x	20.x	25.xi	24
13b	2.xi	3.xi	30.xi	26
14b	16.xi	17.xi	6.xii	28
15b	7.xii	8.xii	20.xii	31

Respiration Rate.—The mean rates of oxygen uptake and carbon dioxide production for the fifteen samples of mature apples are shown in Figs. 5 and 6 respectively. Most
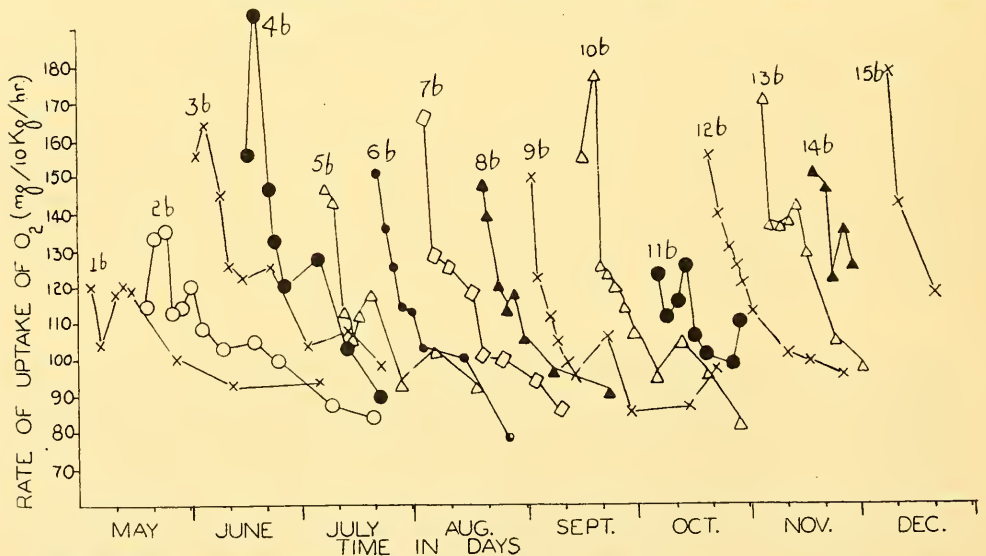

Fig. 5.—Graph showing the mean rates of uptake of oxygen (per unit weight) for each of the thirteen samples of mature Granny Smith apples taken during 1943.

of the early curves for rate of output of carbon dioxide by mature fruits showed distinct peaks during the first week after removal from store, after which they fell more or less steadily throughout the period of observation. The peaks occurred in the curves for most individuals within each sample. The curves for mean rate of uptake of oxygen frequently showed no peaks. When a peak was present, it was usually less distinct than the corresponding peak for output of carbon dioxide. This was due to the fact that fewer individuals showed peaks for uptake of oxygen than for output of carbon dioxide. Some individuals showed peaks for both rates, some showed no peaks, and the rest showed a peak for rate of output of carbon dioxide but no peak for uptake of oxygen. In the last type, the respiratory quotient was initially less than 1, but rose to approximately 1 when the peak was observed. No individual showed a significant peak for rate of uptake of oxygen without a corresponding peak for output of carbon dioxide.
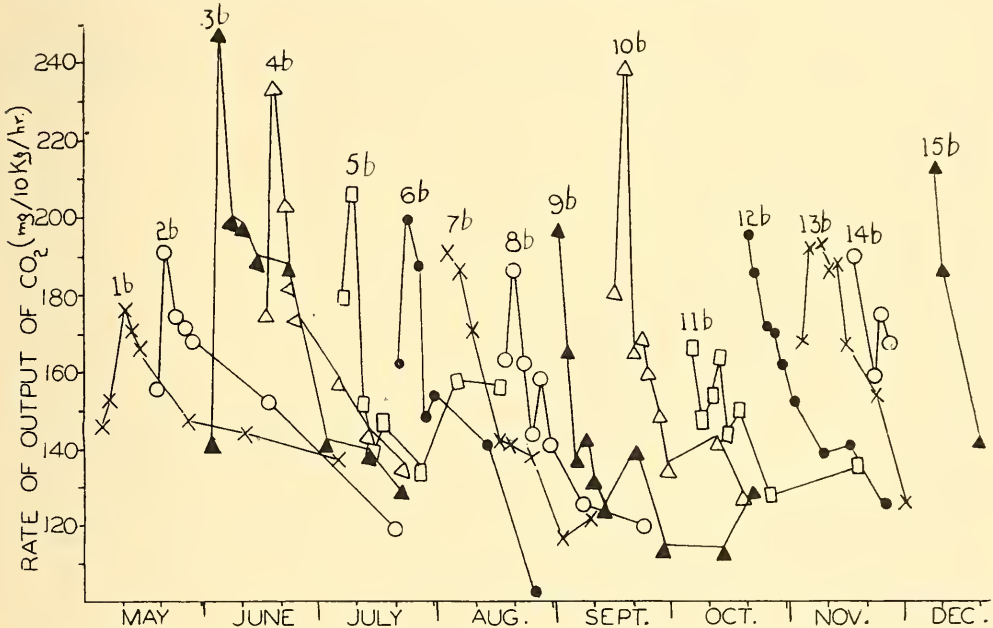

Fig. 6.—Graph showing the mean rates of output of carbon dioxide (per unit weight) for each of the thirteen samples of mature Granny Smith apples taken during 1943.

The mean initial rates of uptake of oxygen and output of carbon dioxide showed no definite trends.

Mean values for the respiratory quotient are shown in Fig. 7. Only the first few values for each sample are included in the figure as no change occurred subsequently. The initial value was frequently less than 1 on the first day after removal from store but had risen to approximately 1 when the second reading was taken. The reason for the low initial respiratory quotient is not known. Possible inaccuracies which might
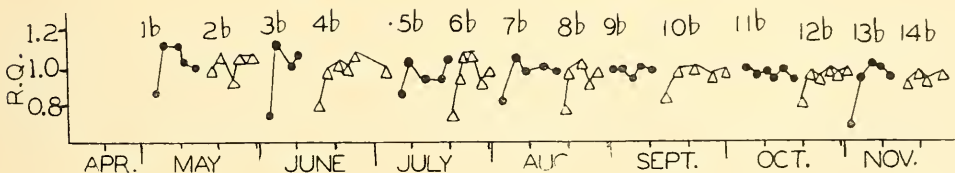

Fig. 7.—Graph showing the mean values for the respiratory quotient in each of the samples of mature Granny Smith apples taken during 1943. The curves have been curtailed, as no further change was observed in the respiratory quotient of any of the samples.

occur as a result of incomplete temperature equilibration (e.g., inaccuracies due to liberation of dissolved or adsorbed carbon dioxide at the higher temperature) would tend to increase the respiratory quotient, not decrease it.

Composition of the Internal Atmosphere.—The mean concentrations of oxygen and carbon dioxide present in the internal atmospheres of the fifteen samples are shown in Fig. 8. In the first seven samples the internal oxygen concentration fell throughout the period of observation. The corresponding concentration of carbon dioxide rose slightly in samples 1*b* to 4*b* but remained steady or fell in samples 5*b* to 7*b*. In samples 8*b* and 9*b* the changes observed in the internal oxygen concentration were relatively slight; the corresponding concentration of carbon dioxide fell; in the last samples (10*b* to 15*b*) oxygen concentration either remained constant or rose slightly. The corresponding concentration of carbon dioxide remained constant or fell.
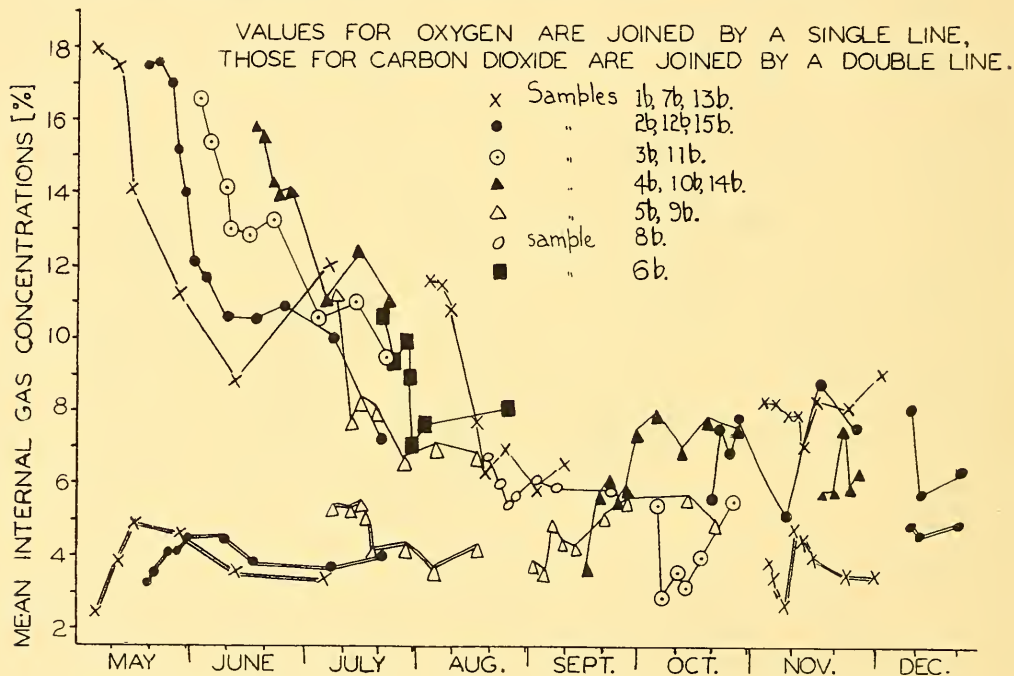

Fig. 8.—Graph showing the mean internal oxygen concentrations for all the samples of mature Granny Smith apples and the mean internal carbon dioxide concentrations for typical early and late samples of mature Granny Smith apples of the 1943 season.

The mean initial oxygen concentration decreased from sample to sample until 10*b* was reached. The initial oxygen concentrations of the later samples were higher than that of 10*b*, those of 13*b* and 15*b* being the highest. The corresponding concentrations of carbon dioxide tended to rise from sample to sample until 10*b* was reached. In this sample the initial concentration of carbon dioxide was higher than that of oxygen. In the later samples no further rise was observed.

Resistance of the Skin to Gaseous Diffusion.—The mean resistances of the skin to the diffusion of oxygen and carbon dioxide are shown in Fig. 9. The mean resistance to the diffusion of oxygen (r_o) increased markedly in all the early samples (1*b* to 9*b* inclusive). In sample 10*b* it remained constant; in later samples it rose, although generally less steeply than in the early samples. The mean initial value for r_o tended to rise from sample to sample, reaching a maximum with sample 10*b*. For later samples it tended to fall, the lowest value being that of sample 13*b*.

The mean resistance of the skin to the diffusion of carbon dioxide (r_c) increased in most of the early samples. In later samples it remained constant. The initial values
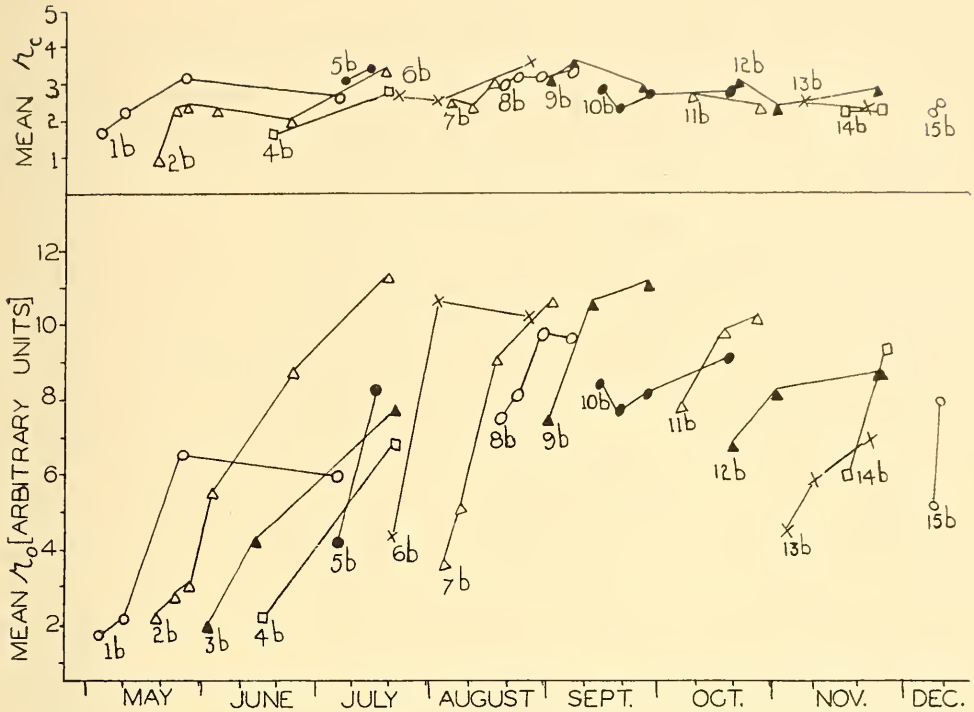
Fig. 9.—Graph showing mean resistances to the diffusion of oxygen (r_o) and carbon dioxide (r_c) for each of the samples of mature Granny Smith apples taken in 1943. The arbitrary units used here are not the same as those used in Fig. 4.

for r_c were highest in the samples which had the highest initial values for r_o. The regression coefficient of r_c on r_o was 1·14 and was significant (P<0·02).

<center>EXPERIMENTAL RESULTS.   C.</center>

The Behaviour of Fruits in an Atmosphere of Pure Oxygen.—During 1943, six experiments were carried out to determine the rate of output of carbon dioxide of immature and mature apples when surrounded by an atmosphere of pure oxygen.

In the first experiment, twelve immature fruits were used which were comparable with those of sample $8a$. Table 3 shows the rates of output of carbon dioxide when the fruits were in air and when they were in pure oxygen.

It is clear that, in fruits at the particular stage of development used, increased oxygen supply had no effect on the rate of output of carbon dioxide. These fruits were of the same age as those of sample $8a$, in which the mean internal concentrations of carbon dioxide and oxygen were about 2·5% and 18·5%, respectively, and the mean values for r_c and r_o were very low (less than 2 units, whether calculated on respiration per unit weight or on respiration per unit surface).

The other five experiments with pure oxygen (numbers 2 to 6, inclusive) were carried out with mature apples at 18·3°C. In the second experiment fruits from samples $1b$ and $4b$ were used. The experiment was begun on 28.vi.43. Sample $1b$ had been held at 18·3°C. since 4.v.43 and sample $4b$ had been at 18·3°C. since 16.vi.43. Table 4 shows the rates of output of carbon dioxide under various conditions.

Increased oxygen supply did not have a great effect on fruit 1 of sample $4b$, but the rates of output of carbon dioxide of the other fruits rose considerably. Thus, even in fruits which had been held at 18·3°C. for eight weeks (sample $1b$), oxygen supply was apparently limiting respiration rate. It appears that the substrate factor suggested by Blackman and Parija (1928) had not assumed control. The values for r_o for fruits 1 and 2 of sample $1b$ and fruit 1 of sample 4 were high (12·0, 7·4 and 6·4 units, respectively,

TABLE 3.

First Experiment with Pure Oxygen; Immature Fruits of the Same Age as those of Sample 8a.

Date.	No. of Fruit.	Surrounding Atmosphere.	Output of CO_2 (mg./10 Kg./hr.)	No. of Fruit.	Surrounding Atmosphere.	Output of CO_2 (mg./10 Kg./hr.)
31.iii.43	1	Air.	133	7	Air.	144
11.25 a.m.–	2		130	8		129
4.10 p.m.	3		115	9		112
	4		119	10		137
	5		163	11		139
	6		140	12		143
31.iii–1.iv Overnight.	1 to 6	Oxygen.	Not observed.	7 to 12	Air.	Not observed.
1.iv	1	Oxygen.	126	7	Air.	106
10.30 a.m.–	2		115	8		199
3.30 p.m.	3		96	9		115
	4		93	10		118
	5		115	11		122
	6		108	12		125
1–2.iv	1	Oxygen.	110	7	Air.	111
3.30 p.m.–	2		105	8		102
10.30 a.m.	3		102	9		100
	4		90	10		99
	5		100	11		116
	6		98	12		119
2.iv	1	Oxygen.	136	7	Air.	122
10.35 a.m.–	2		111	8		118
4.5 p.m.	3		122	9		115
	4		113	10		118
	5		103	11		133
	6		108	12		130

TABLE 4.

Second Experiment with Pure Oxygen; Mature Fruits of Samples 1b and 4b.

Date.	Sample No. and No. of Fruit.	Surrounding Atmosphere.	Output of CO_2 (mg./10 Kg./hr.)	% Increase in Rate of Output of CO_2.	Sample No. and No. of Fruit.	Surrounding Atmosphere.	Output of CO_2 (mg./10 Kg./hr.)	% Increase in Rate of Output of CO_2.
28.vi.43	1b–1	Air.	105	—	1b–3	Air.	116	—
	1b–2		108	—	1b–4		105	—
	4b–1		153	—	4b–3		153	—
	4b–2		166	—	4b–4		—	—
28–29.vi	1b–1 to 4b–2	Oxygen.	Not observed		1b–3 to 4b–4	Air.	Not observed.	
29.vi	1b–1	Oxygen.	126	20	1b–3	Air.	113	—
9.35 a.m.–	1b–2		102	0	1b–4		98	—
3.35 p.m.	4b–1		135	—	4b–3		112	—
	4b–2		151	—	4b–4		129	—
29–30.vi	1b–1	Oxygen.	147	39	1b–3	Air.	118	2
3.35 p.m.–	1b–2		135	25	1b–4		111	6
noon.	4b–1		167	10	4b–3		130	−15
	4b–2		201	12	4b–4		131	—
30.vi–1.vii	1b–1	Oxygen.	149	42	1b–3	Air.	121	4
4 p.m.–	1b–2		130	17	1b–4		102	4
9.45 a.m.	4b–1		170	10	4b–3		129	−15
	4b–2		185	12	4b–4		132	—
1–2.vii	1b–1	Oxygen.	131	25	1b–3	Air.	117	0
9.45 a.m.–	1b–2		158	21	1b–4		116	2
9.45 a.m.	4b–1		157	3	4b–3		140	−9
	4b–2		199	20	4b–4		136	—

calculated on respiration per unit weight). The value for fruit 2 of sample 4 was not obtained.

In the third experiment, fruits 1, 2, 3 and 4 of sample 1b were used again, this time with fruits 1, 2, 3 and 5 of sample 7. Sample 1b had been out of store for fourteen weeks and sample 7b had been out of store for one week. Air was passed through all the vessels on 10–11.viii.43 and oxygen was supplied to four of the fruits from the morning of 11.viii. The rates of output of carbon dioxide by these four fruits rose slowly until 13.viii., when the experiment was concluded. Table 5 gives the most relevant data.

TABLE 5.

Third Experiment with Pure Oxygen; Mature Fruits of Samples 1b and 7b.

Date.	Sample No. and No. of Fruit.	Surrounding Atmosphere.	Output of CO$_2$ (mg./ 10 Kg./hr.)	% Increase in Rate of Output of CO$_2$.	Sample No. and No. of Fruit.	Surrounding Atmosphere.	Output of CO$_2$ (mg./ 10 Kg./hr.)	% Increase in Rate of Output of CO$_2$.
10–11.viii.43	1b–1	Air.	123	—	1b–3	Air.	126	—
	1b–2		107	—	1b–4		107	—
	7b–1		162	—	7b–3		147	—
	7b–2		173	—	7b–5		168	—
13.viii	1b–1	Oxygen.	172	40	1b–3	Air.	128	0
	1b–2		139	30	1b–4		116	8
	7b–1		224	39	7b–3		140	−5
	7b–2		201	16	7b–5		169	0

Even after being held at 18·3°C. for fourteen weeks, the fruits of sample 1b showed a considerable rise in respiration rate when pure oxygen was supplied.

In the fourth experiment, fruits of sample 12b, which had been out of store for two weeks, were used. Table 6 shows the increase which occurred in the rate of production of carbon dioxide. The mean value for r_0 in these fruits was 7·9 units.

TABLE 6.

Fourth Experiment with Pure Oxygen; Mature Fruits of Sample 12b.

Date.	No. of Fruit.	Surrounding Atmosphere.	Output of CO$_2$ (mg./ 10 Kg./hr.)	% Increase in Rate of Output of CO$_2$.	No. of Fruit.	Surrounding Atmosphere.	Output of CO$_2$ (mg./ 10 Kg./hr.)	% Increase in Rate of Output of CO$_2$.
2–3.xi.43	1	Air.	163	—	4	Air.	160	—
12.15 p.m.–	2		145	—	5		151	—
9.15 a.m.	3		140	—				
3–4.xi	1	Oxygen.	201	23	4	Air.	169	5·6
9.30 a.m.–	2		154	6·2	5		141	−6·6
10 a.m.	3		145	3·6				
4–5.xi	1	Oxygen.	207	27	4	Air.	154	−3·8
10 a.m.–	2		164	13·1	5		136	−10
9.30 a.m.	3		162	15·7				
5.xi	1	Oxygen.	214	30·7	4	Air.	145	−9·4
9.30 a.m.–	2		176	21·5	5		131	−13·8
4.15 p.m.	3		169	20·8				

The fifth experiment was similar to the fourth, but the fruits used were those of sample 14b, which had been held at 18·3°C. for nearly two weeks. The mean value for r_0 was 9·5 units. When three of the fruits had been in oxygen for 48 hours the highest increase observed in the rate of output of carbon dioxide was 14·4% for apple 1. This

was not a significant response, since the corresponding rate for fruit 5 in air rose 16.7% during the same period. When the three fruits had been in oxygen for 72 hours, the rise in respiration rate was only slightly higher than the rise in respiration rate of fruit 5 which was still in air (see Table 7).

TABLE 7.

Fifth Experiment with Pure Oxygen ; Mature Fruits of Sample 14b.

Date.	No. of Fruit.	Surrounding Atmosphere.	Output of CO_2 (mg./ 10 Kg./hr.)	% Increase in Rate of Output of CO_2.	No. of Fruit.	Surrounding Atmosphere.	Output of CO_2 (mg./ 10 Kg./hr.)	% Increase in Rate of Output of CO_2.
29–30.xi.43	1	Air.	125	—	4	Air.	182	—
12.30 p.m.–	2		149	—	5		114	—
9.30 a.m.	3		144	—				
30.xi–1.xii	1	Oxygen.	130	4	4	Air.	176	−3·3
9.35 a.m.–	2		165	10·8	5		150	29
9.20 a.m.	3		159	10·4				
1–2.xii	1	Oxygen.	143	14·4	4	Air.	172	−5·5
9.20 a.m.–	2		161	8·0	5		133	16·7
9.20 a.m.	3		158	9·7				
2–3.xii	1	Oxygen.	148	17·0	4	Air.	170	−6·6
9.20 a.m.–	2		180	20·8	5		134	17·5
9.20 a.m.	3		187					

The sixth experiment was similar to the fourth and fifth, using fruits of samples 14b and 15b, which had been at $18.3°C.$ for three and a half weeks and five days, respectively. The mean value for r_0 was 8·2 units. Owing to trouble with the manipulation of the oxygen supply, no reliable observations were made until the fruits had been in oxygen for more than 50 hours. Table 8 gives the relevant data. The increased oxygen supply had no significant effect on the rate of output of carbon dioxide after 50 hours.

TABLE 8.

Sixth Experiment with Pure Oxygen ; Mature Fruits of Samples 14b and 15b.

Date.	Sample No. and No. of Individual.	Surrounding Atmosphere.	Output of CO_2 (mg./ 10 Kg./hr.)	% Increase in Rate of Output of CO_2.	Sample No. and No. of Individual.	Surrounding Atmosphere.	Output of CO_2 (mg./ 10 Kg./hr.)	% Increase in Rate of Output of CO_2.
13–14.xii.43	14b–2	Air.	110	—	14b–3	Air.	107	—
12.30 p.m.–	15b–2		187	—	15b–4		224	—
10.20 a.m.	15b–3		119	—	15b–5		163	—
14–15.xii	14b–2	Oxygen.	—	—	14b–3	Air.	128	19·6
10.45 a.m.–	15b–2		—	—	15b–4		236	5·4
2.15 p.m.	15b–3		—	—	15b–5		174	6·8
16–17.xii	14b–2	Oxygen.	126	14·5	14b–3	Air.	117	9
4 p.m.–	15b–2		193	3·2	15b–4		214	−4·5
9.45 a.m.	15b–3		180	13·5	15b–5		171	4·9

In all the experiments where the rate of production of carbon dioxide increased in pure oxygen, the normal level had been regained when observations were made two days after the apples were returned to air.

Figure 10 shows the mean rates of output of carbon dioxide for the apples in air and for those in oxygen in each of the six experiments.
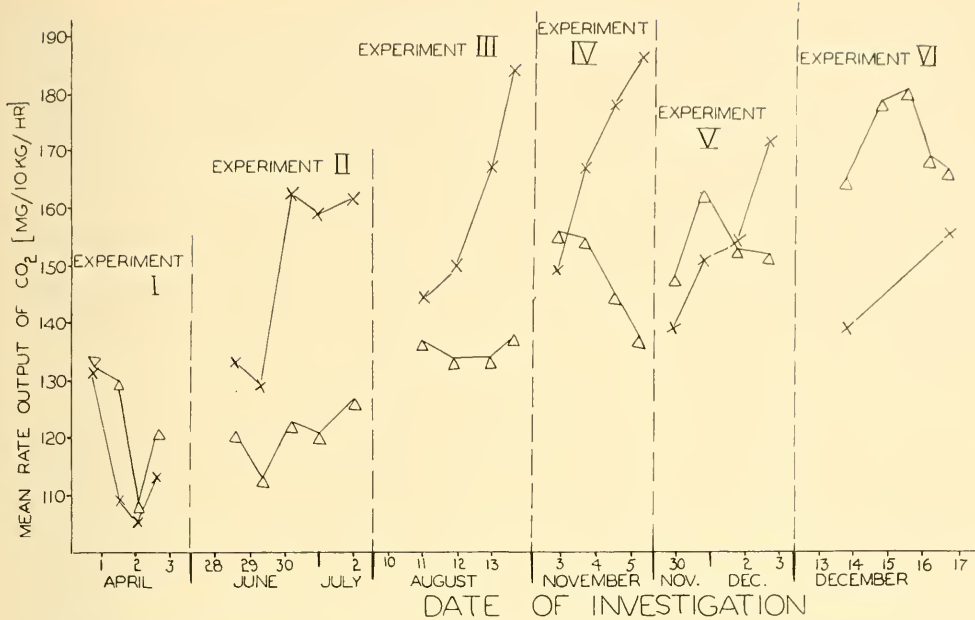
Fig. 10.—Graphs showing the effect of pure oxygen on the rate of carbon dioxide output of Granny Smith apples of the 1943 season at 18·3°C. In each experiment crosses represent values for fruits placed in oxygen after the first observation had been made, and triangles represent values for fruits held in air throughout the period of observation. Immature fruits were used in the first experiment; mature fruits which had been in cool store for various periods were used in the other experiments.

Discussion of Results.

Discussion of the Behaviour of Immature Fruits.—When respiration rates were calculated in milligrams of oxygen taken up or carbon dioxide given off per 10 Kg. per hour, a pronounced decrease in respiratory activity was apparent as development proceeded (Fig. 1). When the same rates were calculated in milligrams per fruit per hour (i.e., when an attempt was made to base respiration rate on amount of respiring material present), the decrease in respiratory activity was not evident. The method of calculation of the respiration rate using the whole fruit as a unit is, of course, open to the objection that different fruits contain different numbers of cells and different amounts of protoplasm. However, in the absence of further data, the figures used should give a reasonable approximation for the comparison of the rates of respiration per unit respiring material for fruits at different stages of development. In future work a closer approximation could be obtained by calculating respiration rate on the basis of total protein or total nitrogen present in each fruit.

The decrease in initial respiration rate per unit weight as development proceeds has been observed in Jonathan apples by Shaw (1942), and in McIntosh and Northern Spy apples by Krotkov (1941). Shaw observed a rise in rate of uptake of oxygen extending over a short time after fruit setting, followed by a decrease as the fruit developed. Krotkov observed a decrease in initial rate of production of carbon dioxide from the time of fruit setting until within a few weeks of picking. As no data have been obtained for Granny Smith apples until some time after fruit setting, it is not known whether the respiration rate per unit weight increases during the initial period in this variety. It is possible that the respiratory trends observed by Shaw and Krotkov might have been quite different had the respiration rates been calculated in units per fruit instead of per unit weight.

Mention has already been made of some of the physiological effects which might result from changes in surface-volume ratio and in the resistance of the skin to gaseous diffusion during development. The effect of changes in the surface-volume ratio could

not be directly observed. It has been shown that even when the surface-volume factor is eliminated from the calculations, appreciable changes occur in the resistance of the skin of the fruit to the diffusion of oxygen and carbon dioxide. Changes in surface-volume ratio and changes in the resistance of the skin could only affect the respiration rates by modifying the concentrations of oxygen and carbon dioxide in the internal atmosphere. The observed changes in the internal atmosphere were small. In addition, the lack of any change in the respiration rate when immature fruits were held in pure oxygen proves that at that stage the respiration rate was not limited by oxygen supply. The resistance changes observed in the immature fruit could not, therefore, have had any effect on the respiration rates.

The shapes of the respiration curves for immature fruits are probably influenced by other factors, and it would be of little use to speculate further until more information has been obtained.

Comparison of the Results for Mature Fruits of 1943 with those of Previous Years.

Rates of Output of Carbon Dioxide.—The shape of the curves for early samples for mean rate of output of carbon dioxide (Fig. 4) is somewhat different from that of the corresponding curves obtained during 1941 and 1942 (Hackney, 1943). The latter, although showing fluctuations from time to time, did not exhibit any disturbance great enough or of long enough duration to be compared with the climacteric rise observed in some varieties of apples and in other fruits by Blackman and Parija (1928), Wardlaw and Leonard (1936) and other workers. Many of the curves obtained during 1943 showed a distinct increase in the rate of output of carbon dioxide within the first week after removal from store. In 1943 the first observation was always made on the first day after removal from store and the second observation was generally made two days later, no observation being made on the second day after removal from store. The 1941 experiments were begun similarly, but in 1942 the first observation was not generally made until the second day after removal from store (Hackney, 1943). In the early samples for 1943 the first reading was often considerably lower than the second. In corresponding samples for 1941 and 1942 the first reading was generally the highest observed. It is possible that the respiration rates were lower on the first day after removal from store in 1942, as in 1943, and that the lack of observations earlier than the second day after removal from store was responsible for the difference in the shape of the curves. If the first reading were omitted for each of the 1943 samples, the curves would be very similar to those obtained in 1941. It is probable that the 1941 fruits were more mature than the 1943 fruits at the time of picking, and had passed through the phase of rising respiration rate while still on the tree.

Composition of the Internal Atmosphere.—The changes which took place in the composition of the internal atmospheres of the early samples of mature fruits in 1943 were very similar to those which took place in corresponding samples of the 1941 and 1942 seasons (Hackney, 1943). The later samples for 1943 differed from those of 1941 and 1942 in that the mean concentration of oxygen in the internal atmosphere did not generally rise during the period of observation. Even when an increase was observed (samples 9 and 10), its magnitude was relatively small (3.8% to 7.7%) compared with those observed in previous years (6% to 15% in 1941 and 5% to 13% in 1942).

Resistance of the Skin to Gaseous Diffusion.—As in 1941 and 1942 (Hackney, 1943), the mean values for r_o in the early samples increased throughout the period of observation. But in 1941 and 1942, r_o remained constant or decreased in the later samples, whereas it continued to increase in the corresponding samples for 1943. Number 10 was the only 1943 sample in which r_o did not increase.

As in 1941 and 1942, changes in r_c were much less noticeable than changes in r_o. A slight increase over the period of observation was generally observed in the early samples. In the later samples, r_c remained approximately constant.

Respiratory Quotient.—The respiratory quotient was not measured in 1941 or 1942. In order to calculate r_o in the previous papers of this series, the value for the respiratory quotient was assumed to be approximately 1. The values obtained for the respiratory

quotient during 1943 indicate that in normal fruits any variations from this value are so slight as not to affect the shape of the curves for r_o. In 1941–42, it was suspected that a small amount of anaerobic respiration occurred in air when the internal oxygen concentration was low. If that was true during 1943, the amount of carbon dioxide produced anaerobically must have been too small to alter the respiratory quotient noticeably.

GENERAL DISCUSSION OF BEHAVIOUR OF MATURE FRUITS.

Respiration Rates.—The data obtained for mature fruits during 1943 present problems which were not indicated in those obtained during 1941–42. The occurrence of what is possibly analogous to the 'climacteric rise' in the early curves for rate of output of carbon dioxide is an important new feature. The term 'climacteric rise' was employed by Kidd and West (1930) to denote the rise in respiration rate which marked the onset of ripening and senescence in Bramley's Seedling apples. It has since been adopted by many plant physiologists to denote similar phenomena in other fruits. In most fruits the beginning of the climacteric rise coincides with the first appearance of ripening colour, but this rule is not without exceptions. Granny Smith apples removed from store generally colour with no accompanying rise in respiration rate. Respiratory peaks may occur naturally, or be induced artificially, for a number of different reasons (e.g., artificial increase in oxygen supply). Many fruits have more than one respiratory peak (natural, or artificially induced) during the period of observation. For instance, three peaks occurred in sample 1, 1943, the first occurring in air twelve days after removal from store, the second and third being induced in pure oxygen eight and fourteen weeks, respectively, after removal from store. The causes underlying these peaks were different, and the onset of colouring was not associated with any of them. Thus care must be taken to guard against the indiscriminate use of the term 'climacteric rise' to denote peaks due to entirely different causes.

No explanation is at present available for the difference in the respiratory quotient of the 1943 Granny Smith apples before and after the respiratory peak. The respiratory quotient was frequently less than 1 on the first day after removal of the fruits from store but had risen to approximately 1 when the peak was observed. The reason for the low initial value is not known. When a fruit is removed from a low temperature to a higher one, various physical and chemical changes occur suddenly, and a number of hours must elapse before the equilibrium state for the new temperature is reached. Blackman and Parija (1928) studied the respiratory behaviour of Bramley's Seedling apples on removal from 2·5°C. to 22°C. It was found that the rate of output of carbon dioxide rose to a peak soon after the change was made; it then fell and seemed to have reached its new equilibrium 20 hours after being placed at 22°C. It is possible that the Granny Smith apples used in 1943 had not reached their new respiratory equilibrium when the first observation was made 25 hours after removal from store. If this was so, it is difficult to understand why the first reading was lower than, not higher than, later readings. All the physical changes usually thought to be associated with increases in temperature of the fruit (e.g., liberation of dissolved or adsorbed carbon dioxide) should tend to increase the rate of output of carbon dioxide rather than to decrease it.

Whatever the cause of the low initial respiratory quotient, it is improbable that the peak observed in the rate of output of carbon dioxide was solely due to the temperature change at the time of removal from store, since no such peaks were observed in the early samples for 1941, when the temperature changes were the same. There was apparently some real physiological difference between the 1943 fruits and those of previous years.

The Relationship of Respiration Rate to Oxygen Supply.—The strong positive correlation observed between internal oxygen concentration (governed by the resistance of the skin to gaseous diffusion) and respiration rate, in samples removed from store early in the year, has been fully discussed for 1941–42 in a previous paper (Hackney, 1943). The same correlation was observed in those of the 1943 samples which had not been in store for more than three months. In immature fruits, where the resistance of the skin to gaseous diffusion was low, the rate of production of carbon dioxide was not

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affected when the fruit was held in an atmosphere of pure oxygen; from which it may be concluded that oxygen supply was not limiting respiration rate in these fruits. When early samples of mature fruits were held in an atmosphere of pure oxygen, the rates of production of carbon dioxide increased markedly, even in fruits which had been out of store for many weeks, and whose appearance and flavour suggested that they might have been poor in substrate. This fact, together with the positive correlation observed between internal oxygen concentration and respiration rate in the early mature samples, provides strong support for the theory that oxygen supply limits the respiration rate of the fruit during the greater part of its storage life. When the very late samples (late Nov.–Dec.) were held in pure oxygen, the respiration rate showed little or no change. It was suspected from the 1941–42 data that oxygen supply ceased to be the main factor limiting respiration rate late in the year. In late samples for 1941–42 the internal oxygen concentration rose as respiration rate declined, the resistance of the skin to gaseous diffusion remaining constant. In corresponding samples for 1943 the internal oxygen concentration did not rise as respiration decreased. This was due to the fact that the resistance of the skin to the diffusion of oxygen continued to increase.

The data obtained during 1943 lend strong support to the theory suggested by the data of 1941–42, namely, that oxygen supply is the main factor limiting respiration rate during the greater part of the storage life of the Granny Smith apple. The depressant effect of artificial lowering of the oxygen supply on respiration has been studied by several investigators, from Parija (1928) to Platenius (1943), but the possibility of the respiration rate being limited by natural restriction of oxygen supply is a comparatively recent discovery (Wardlaw and Leonard, 1936). The part played by oxygen supply in the Granny Smith apple may be summarized thus. In the immature fruits, where the resistance of the skin to gaseous diffusion is low, increased oxygen supply has no effect on respiration rate. At this stage, respiration rate is limited by some other factor, such as substrate supply or enzyme activity. In mature fruits which have not been in store for more than three or four months the rate of respiration is probably limited by oxygen supply. It is not directly limited by substrate scarcity or by lack of enzyme capacity, since increased oxygen supply results in an increased rate of respiration. In mature fruits which have been in store for six months or more, oxygen supply has probably ceased to be the main limiting factor. Respiration rate is then limited by some other factor, possibly the substrate scarcity suggested by Blackman and Parija (1928), possibly the deterioration of the enzyme system with advancing age of the fruit.

Summary.

Two series of experiments have been carried out on the respiratory behaviour of Granny Smith apples of the 1943 season at 18·3°C. For the first series, the fruits were picked at various stages of development, and observations were made on respiration rates and composition of the internal atmosphere during the first two weeks after picking. For the second series, fruits of commercial maturity were stored at 0°C. Samples were taken from store at fortnightly intervals, and observations were made on respiration rates and composition of the internal atmosphere for at least four weeks. The effects of an atmosphere of pure oxygen on the respiration rate have been studied.

In immature fruits the initial respiration rates per unit weight decreased as development proceeded, until about six weeks before the date of commercial picking. When respiration rates were calculated in units per fruit, instead of per unit weight, this decrease was not evident. The respiration rates for individual fruits generally decreased throughout the period of observation. The respiratory quotient was approximately 1 throughout development. The internal oxygen concentration fell slightly and the internal carbon dioxide concentration rose slightly as development proceeded. The calculated resistances of the skin to the diffusion of oxygen and carbon dioxide increased slightly during development.

The behaviour of mature fruits which had not been in store for more than four months differed from that of fruits which had been in store for longer periods. In early samples the mean respiration rate (carbon dioxide output) generally rose to a peak soon after removal from store. After the peak was attained, the respiration rate

decreased throughout the period of observation. The decreasing respiration rate was associated with a decreasing internal oxygen concentration and an increasing resistance of the skin to the diffusion of oxygen. The respiratory quotient was frequently less than 1 on the day after removal from store but had risen to 1 two days later.

In the samples which had been in store until late in the year respiratory peaks did not occur, the respiration rates decreasing throughout the period of observation. The internal oxygen concentration was low on removal from store, and little change occurred subsequently. The resistance of the skin to the diffusion of oxygen was high initially and generally increased at 18·3°C.

Results of experiments in which apples were held in pure oxygen indicate that oxygen supply limits the respiration rate of mature apples which have not been in store for more than four months, but does not limit the respiration rate of either immature apples or apples which have been in store for more than four months.

The implications of these results are discussed in detail.

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